

AGILENT BIOANALYZER

RNA ASSAY KIT

RNA 6000 Nano Kit
(Cat# 5065-4476)

RNA Chips

25 RNA Chips per box
2 Electrode Cleaners

RNA 6000 Nano Assay Reagents & Supplies

RNA Dye Concentrate (Blue)
RNA 6000 Nano Marker (Green)
RNA Gel Matrix (Red)
3 Spin Filters

Syringe Kit

1 Syringe

Additional (not supplied)

RNA 6000 ladder (Ambion cat# 7152)
RNAseZAP for electrode decontamination (Ambion cat# 9780)
Microcentrifuge tubes (RNAse-free): 1.5ml for gel
preparation
Vortex mixer
MJ research 0.6 ml tubes for incubation

Essential Practices

- 1) When dispensing into the chip wells the pipette tip must be inserted to the bottom of the well. Placing the pipette at the edge of the well leads to bubbles and poor results.
- 2) Keep all reagent and reagent mixes refrigerated at 4°C when not in use.
- 3) Allow all reagents to equilibrate to room temperature (30 minutes) before use.
- 4) Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.

- 5) Prepared chips must be used within 5 minutes to avoid evaporation and poor results.
- 6) Vortex all samples in sample buffer thoroughly before loading on chip.
- 7) Use a new syringe with each new kit.
- 8) Use a new cleaning chip with each new kit.
- 9) The RNA ladder must be heat denatured before use. Aliquot ladder into a Rnase-free microcentrifuge tube and heat at 70°C for two minutes. Put on ice.

Decontaminating the Electrodes

Open the lid to the bioanalyzer.

Pull out present electrode assembly and insert electrode assembly used only for RNA.

Clean electrodes as described.

- 1) Slowly fill one of the wells of an electrode cleaner chip with 350 µl RNaseZAP.
- 2) Open the lid and place in the chip slot.
- 3) Close the lid and leave it closed for 1 minute.
- 4) Open the lid and remove the electrode cleaner. The same cleaner chip can be reused for all the chips in the kit. Remove the RNaseZAP from the cleaner chip at the end of the day.
- 5) Slowly fill one of the wells of another electrode cleaner with 350 µl RNase-free water.
- 6) Place electrode cleaner in the Agilent 2100 Bioanalyzer.
- 7) Close the lid and leave it closed for 10 seconds. Open the lid and remove the electrode cleaner chip. Wait another 10 seconds for the water on the electrodes to evaporate.

Gel-Dye Mix Notes

- 1) Store the gel-dye mix at 4°C when not in use for more than an hour.
- 2) Use the gel-dye mix within three days (max. one week) of preparation.

Protect the gel-dye mix from light or the dye will degrade reducing the signal intensity.

- 3) Allow the gel-dye mix to equilibrate to room temperature (30 minutes) before use.
Protect the gel-dye mix from light while bringing it to room temperature.

Preparing the Gel-Dye Mix

- 1) In top receptacle of provided spin filter place 400 μ l of RNA gel matrix (Red). Make sure this tube has come to room temperature and is thoroughly vortexed (contains DMSO).
- 2) Place spin filter in a microcentrifuge and spin at 1500g +/- 20% for 10 minutes (5,000 RPM in Eppendorf microcentrifuge).
- 3) Discard spin filter, date and label this tube. Do not use after one month.

(steps 4-6 make sufficient mix for 4 chips)

- 4) Place 130 μ l of this filtered RNA gel matrix in a new 1.5ml microfuge tube. Add 2 μ l of RNA dye concentrate (Blue).
- 5) Cap the tube and vortex thoroughly and inspect to ensure proper mixing of gel and dye.
- 6) Cover with foil. Date tube, use within three days (one week max.)

Sample Preparation

- 1) Thaw RNA samples on ice.
- 2) Dilute samples to ~100ng/ μ l.
- 3) Dispense 1 μ l of RNA in each tube. Also add 1 μ l of ladder to the appropriate tube.
- 4) Heat denature samples at 70°C for at least 2 minutes. Put on ice.
- 5) Label PCR tubes appropriately and add 5 μ l RNA 6000 Nano Marker (Green).
- 6) Vortex well and spin down.

Loading the Gel-Dye Mix

- 1) Take a new chip out of its sealed bag.
- 2) Place the chip on the Chip Priming Station. Make sure the station base plate is in position C before loading.

- Make sure the adjustable clip is in the upper position and the syringe is pulled up to the 1.0 mark.
- 3) Draw up 9.0 μ l of the gel-dye mix with a pipette.
 - 4) Place the tip of the pipette at the bottom of the well marked G (dark letter) and dispense the gel-dye mix.
 - 5) Make sure that the plunger is at 1 ml, then close the Chip Priming Station (must hear a click).
 - 6) Press the plunger until it is held by the syringe clip.
 - 7) Wait for exactly 30 seconds and then release the plunger with the clip release mechanism.
 - 8) Pull the plunger back to the 1 ml position.
 - 9) Open the Chip Priming Station.
 - 10) Turn over the chip to check for air bubbles. They will be obvious. Otherwise the back of the chip should look dark and homogeneous.
 - 11) Pipette 9.0 μ l of the gel-dye mix in each of the wells marked G (light letter).

Loading a Prepared Chip

- 1) Add 6 μ l samples to each well in a prepared chip (see loading the gel-dye mix section), starting with the ladder. Use well #1 for a positive control (see Jeff Tucker). Samples must be at room temperature to run.
- 2) Place ladder in the well marked with a picture of a ladder.
- 3) All wells must be filled with Nano Marker sample buffer, even when running less than 12 samples.
- 4) Be sure to keep pipette all the way down to the bottom of the well when dispensing.

Inserting the Chip in the Bioanalyzer

- 1) Open the lid to the Bioanalyzer.
- 2) Place the chip into the receptacle.
- 3) The chip fits one way. Don't use force.
- 4) Carefully close the lid.
- 5) The software screen will show a picture of an icon of a chip if it recognizes it.

Running the Software

- 1) Select the appropriate assay from the Assay menu (ie. Eukaryote total RNA, mRNA, Prokaryote total RNA, DNA and so forth).
- 2) Click on the START icon.
- 3) The START dialogue box appears. The type of assay will be designated in the window. The file profile will say: BioSizing. Change this run title to the chip number and your initials (ex. chip58DD). The extension to this file name automatically includes the date and time.
- 4) Click the start button to begin the assay.
- 5) Complete the sample name table when it pops up and press OK.
- 6) If the error message "Voltages out of range" occurs, there is not enough liquid in the wells. Prepare another chip.
- 7) After the run begins, the start button on the screen changes to a STOP. You may click on it if you need to end the run for any reason.
- 8) To view results for individual wells as data is acquired or after the run is finished, click a well in the chip, a single well displayed on the large 12-well display, or a lane in the gel. Data regarding that well appears in a result table at the bottom of the display.
- 9) When the assay is complete, remove the chip from the receptacle of the bioanalyzer and dispose. Clean with RnaseZap and H₂O as before.
- 10) When done for the day, remove RNA electrode and replace with the other electrode assembly.
- 11) Print results for all wells, single image, gel-image, and individual electropherograms if desired. Fill out log book and store results in provided binder.